梨葉緣焦枯病

蘇秋竹[1,3]、馮鈞育[1]、高清文[1]、曾國欽[2]、徐世典[2]

[1] 行政院農業委員會農業藥物毒物試驗所

[2] 國立中興大學植物病理學系

[3] 聯絡作者，電子郵件信箱：auba@tactri.gov.tw

摘　要

梨葉緣焦枯病(pear leaf scorch)為台灣特有之病害，普遍發生於橫山梨(*Pyrus pyrifolia*)栽植區，梨葉緣焦枯病菌之培養性狀及具波浪狀細胞壁(ripple cell wall) 的特性與侷限導管細菌(xylem-limited bacteria) *Xylella fastidiosa* 相同，但梨葉緣焦枯病菌與其他寄主植物來源之 *X. fastidiosa* 菌株間無血清相關性，且以 *X. fastidiosa* 廣效性引子對 272-1-int/272-2-int 及 RST31/RST33 對梨葉緣焦枯病菌菌株進行 polymerase chain reaction (PCR)反應時，亦無任何基因產物。利用隨機引子(OPA11)進行 random amplified polymorphic DNA (RAPD)分析，成功獲得對梨葉緣焦枯病菌獨有核酸片段大小為 1412 bp，從核酸序列中設計得到一組專一性引子對 PLS-F/PLS-R 可增幅出 416 bp 大小之專一性基因產物。此一引子對可直接進行梨葉緣焦枯病田間罹病梨樹組織之 PCR 檢測。利用 RAPD-PCR、enterobacterial repetitive intergenic consensus sequence-PCR (ERIC-PCR)及 BOX-PCR 三種 DNA 指紋圖譜技術分析所得之親緣樹狀圖皆可將 8 種寄主植物之 *X. fastidiosa* 菌株區分為 4 個菌群，分別為：(1)梨菌群、(2)夾竹桃及葡萄菌群、(3)桑椹菌群及(4)胡桃樹、李樹、桃樹及無花果菌群。此等指紋圖譜技術均可有效鑑別梨葉緣焦枯病菌，並顯示其為 *X. fastidiosa* 菌群中唯一之獨立菌系。進一步利用鄰聚法(Neighbor Joining)進行 10 種不同寄主植物之 *X. fastidiosa* 菌株系統親緣分析（phylogentic analysis），由 16S rRNA 序列及 16S-23S rRNA 區間序列所構建的親緣演化樹（phylogentic tree）皆可明顯區分為 2 個菌群，

分別為梨葉緣焦枯病菌菌群及其他 9 種寄主植物 *X. fastidiosa* 菌群，而其他寄主植物 *X. fastidiosa* 菌群皆可再細分 4 個次菌群，分別為(1)夾竹桃菌群、(2)葡萄及桑椹菌群、(3)咖啡及柑橘菌群及(4)胡桃樹、李樹、桃樹及無花果菌群。由以上結果顯示，梨葉緣焦枯病菌為 *Xylella* 屬內僅侷限於台灣獨立的菌群與其他寄主植物 *X. fastidiosa* 菌株間為較低同源性，應可支持梨葉緣焦枯病菌為 *Xylella* 屬內亞種分類地位，甚至可能為新種。本文研發有關侷限導管細菌 PCR 鑑定與偵測技術，未來有潛力應用於梨葉緣焦枯病菌之蟲媒及中間寄主植物快速偵測。合理的管理栽培制度應可減輕梨葉緣焦枯病之嚴重度，剷除罹病株，可減少其感染源，無性繁殖時應避免剪取罹病株之枝條扦插或芽供接穗之用，化學治療方面，注射四環黴素於每年 5 月及 10 月各注射一次，有相當程度之療效，配合增施有機肥可維持病樹正常生產力。

關鍵詞： 梨葉緣焦枯病、侷限導管細菌、聚合酵素連鎖反應、偵測、DNA 指紋圖譜技術、16S rRNA 基因序列、16S-23S rRNA 基因區間序列、系統親緣分析

緒　　言

梨為薔薇科(Rosaceae)梨亞科(Pomoideae)梨屬(*Pyrus*)植物，屬於落葉喬木，原產於亞洲、歐洲及非洲等地，種類極多，全世界栽培地區亦很廣泛，主要栽培種有西洋梨(*P. communis* L.; European pear)和沙梨(*P. pyrifolia* Nakai; Chinese sand pear or Japan sand pear)，目前許多商業化栽培品種(commercial cultivars)皆屬於此二種。台灣梨栽培種為沙梨，依栽培之模式可分為二類：一為高需冷性，如新世紀、幸水、豐水、新興、秋水、福壽、雪梨等品種，需種植於高海拔山區，才能正常萌芽、開花、結果；另為低需冷性品種主要是橫山梨，在低海拔地區即能正常開花結果[50]，最近 30 年來栽植橫山梨之果農流行以人為落葉方式進行產期調節，或以溫帶品種之梨穗寄接橫山梨徒長枝基部，生產高品質之『寄接梨』以提高橫山梨產業之經濟效益[49]。

梨葉緣焦枯病(Pear leaf scorch)為台灣低海拔地區(海拔 1200 公尺以下)栽植園之重要病害，為侷限導管細菌(xylem-limited bacteria) *Xylella fastidiosa* 所引起[48]，梨葉緣焦枯病是 1980 年左右在台灣橫山梨產區發生之新病害，田間罹病梨樹於每年 7 月前後才會呈現典型葉緣焦枯之病徵，提早落葉，逐年形成枝枯並呈現樹勢衰弱之現象，農友根據其病徵俗稱酥葉病，但病因不明，本病於 1990 年才被確認病因[47]，爾後在台灣各地橫山梨產區調查其發生率為 5-43%不等，而高海拔地區如梨山之溫帶梨品種無此病害發生之現象[48, 74]，目前僅知低海拔地區栽培之橫山梨、鳥梨及蜜雪梨品種皆會被感染，於各地栽植區普遍發生，不但造成梨樹葉緣焦枯病之病徵且降低梨樹的樹勢及活力，間接會影響梨樹或寄接梨果實之產量及品質。

X. fastidiosa 可為害許多重要經濟作物，例如葡萄(grapevine)、苜蓿(alfalfa)、桃樹(peach)、李樹(plum)、杏仁(almond)、榆樹(elm)、無花果(sycamore)、桑椹(mulbery)、柑桔(citrus)及咖啡樹(coffee)等[11, 21, 25, 26, 44, 53, 69, 77]，其中許多病害造成產業之嚴重損失，最典型例子如北美葡萄皮爾斯病(Pierce's disease, PD)及南美柑橘斑駁黃化病(citrus variegated cholorsis, CVC)，目前為北美葡萄與巴西柑橘產業主要限制因子[40]。有關 *X. fastidiosa* 之歷史沿革，1892 年時 N. B. Pierce 首先於美國加州地區(California)發現葡萄(*Vitis vinifera* L.)之 PD 病害，由於此病在美國東南部發生相當嚴重，導致由歐洲引進之葡萄樹種大量死亡，造成農民重大損失[32]，爾後發現此病可經由嫁接及蟲媒而傳播，故認為此病應是由病毒(virus)所引起[29]，且經由研究蟲媒而發現位於葡萄園旁之苜蓿(alfalfa)具有高比例之苜蓿矮化病(alfalfa dwarf disease)，推斷此病可能與 PD 之發生有關[28]，而後經證實此二病害為同一病原菌所引起[25]。1971 年 Hopkins 和 Mortensen[39]發現發生 PD 之葡萄病株經四環黴素(tetracycline)處理後可抑制此病之發展，確定病因為原核生物引起。其後 Hopkins 和 Mollenhauer 藉由電子顯微鏡觀察病株導管組織時發現有病原菌之存在，由於其形態及細胞壁構造與 Rickettsiaceae 極為相似，故稱此病原菌為"Rickettsia-like bacteria (RLB)"由於此類病原菌存在於導管又稱侷限導管細菌[38]。然而此病原菌直到 1978 年 Davis 等人[18]利用 JD-1 培養基才可以人工培養，並完成柯霍氏法則，使得有關此菌之研究範

疇更為廣闊。1987 年 Wells 等人(78)根據 DNA 的 G+C 比、血清學(serology)資料、脂肪酸(fatty acid)分析及 16S rRNA gene 等分析結果，指出此病原菌並非 Rickettsiaceae，亦不屬於其它病原細菌，因而建議給予此類侷限導管細菌一新的名字 *Xylella fastidiosa*，以建立一新屬(genus)及此一新屬中唯一的一種(species)。

目前全世界 *X. fastidiosa* 所引起的病害，已被證實的寄主植物超過 145 種(35, 37, 40)，這些來自不同寄主植物之 *X. fastidiosa* 菌株，依交叉接種試驗資料似乎可再細分為病原型(pathotype)，例如來自於葡萄、苜蓿及杏仁之菌株稱為 PD 菌群，來自柑橘及咖啡之菌株稱為 CVC 菌群，來自桃樹及李樹之菌株為同一菌群(66)，而其他寄主植物之 *X. fastidiosa* 菌株目前仍缺乏交叉接種試驗之資料，主要受限於傳統之交叉接種試驗耗時費力，同時 *X. fastidiosa* 為國際檢疫病原菌其菌株不易取得，另一方面 *X. fastidiosa* 不易培養且生長速度緩慢，無法應用傳統之生理生化特性進行種內菌系的鑑別。

梨葉緣焦枯病病徵學

梨葉緣焦枯病罹病株田間病徵於每年 6-7 月(寄接梨果實採收期)開始出現葉緣焦枯，主要特徵是葉片邊緣及葉尖處出現褐化現象，褐化部分會轉為焦枯，有些鄰近健全葉片組織會有黃化現象，褐化之部位會逐漸向葉之中肋方向擴大癒合，焦枯之面積有些僅佔葉之小部分，較嚴重者可達葉面積一半，並會提早落葉，罹病枝條隨著季節發展葉緣焦枯之葉片會漸增加，徒長枝之葉片通常至 8-9 月才開始出現病徵，由下位葉逐漸往上出現葉緣焦枯之趨勢(圖一)；罹病的枝條逐年有明顯生長受阻，罹病株高接之果實較小，影響產量，嚴重者枝幹會逐年枯死，最後整株會逐漸死亡(48)。

X. fastidiosa 引起植物病害主要造成之病徵包括葉片邊緣焦枯、葉片離層產生、枝枯、延緩植物生長和植物活力衰弱導致死亡，其致病機制，一般學者皆認為病原細菌及植物之充塞細胞(tyloses)與膠質(gum)阻塞罹病植物之導管(8, 53, 54, 55, 58)，導致水份壓力(water stress)之產生，促使植物輸送水份之功能失調(3, 22, 23, 33, 53)，此論點亦衍生出植物毒素(phytotoxin)(45, 53, 68)和

生長調節劑失調(growth regulator inbalance)[23, 34]兩個假說(hypotheses)來解釋其致病機制，但確實之原因仍需更多之證據證實。

梨葉緣焦枯病根據電子顯微鏡觀察，病菌聚集在輸送水分之導管內，部份或全部阻塞(圖二)，導致罹病株寄主生理代謝失調，因而每年寄接梨採收期顯現葉緣焦枯之病徵？依據農委會張淑賢處長之分析結果，罹病株表現葉緣焦枯之葉片與正常葉片比較，顯示罹病株有明顯缺鉀現象，但鈣、鎂略有增加，其他營養成份並無差異，但施用鉀肥並無法減輕其病徵之表現，顯示罹病株病徵之表現是病原菌棲息寄主之導管才引起梨樹生理失調所致。

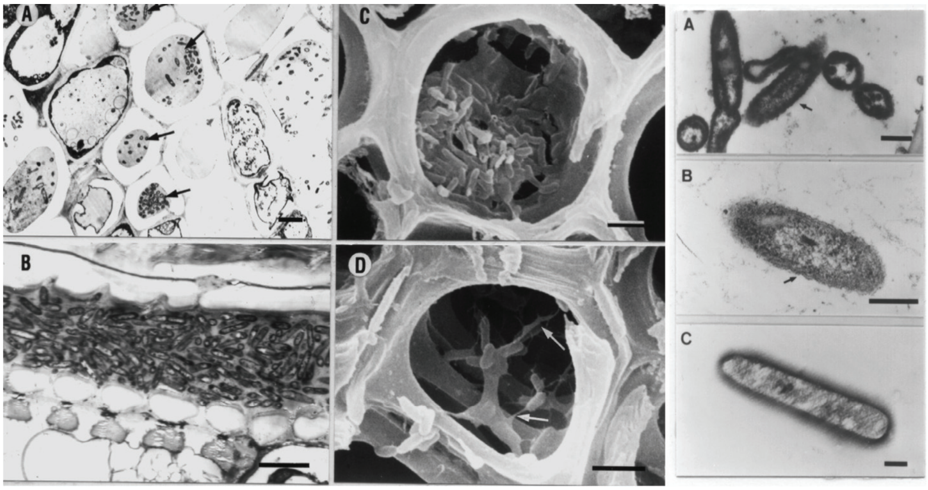
梨葉緣焦枯病原菌特性

梨葉緣焦枯病由侷限導管細菌 *X. fastidiosa* 所引起，細菌為革蘭氏陰性菌 (Gram-negative)、桿狀(rod-shaped)、具有波浪狀細胞壁(ripple-cell wall)，大小約 0.2~0.5×1.1×3.4 µm，病原菌在 PD3、PD2、BYCE、PW 和 CS20 五種培養基皆能生長，但在一般性培養基仍無法生長，在 PD2 固體培養基之菌落形成圓形突起，乳白色，具平滑邊緣，培養 14 天達 0.1~0.2 mm 大小[46, 48]，梨葉緣焦枯病罹病株條全年皆可自枝條莖部汁液分離到病原菌，其月平均分離率 4%~85%，顯示病菌整年皆可存活枝條內，由分離率之變化，枝條內病菌從每年 10 月至翌年 1 月為高峰期，而 3 月至 5 月為低峰期，罹病株葉柄亦可分離到病菌，但需葉緣焦枯病徵出現時才能成功分到病菌，顯示葉片內之侷限導管細菌，在葉片生長中期後才由枝條移動進入葉片內，但根部汁液無法成功分離到病菌[74]。

有關 *X. fastidiosa* 病原菌成功培養沿革，1978 年底 Davis 等人[18]首次利用 JD1 培養基成功分離到造成 PD 及 ALS 之病原菌後，為改進病原菌於培養基上之生長速度和狀況，1980 年 Davis 等人[20]將 JD1 培養基加以修正而發展出培養效果較佳之 PD2 培養基，隨後 1981 年 Davis 等人[19]又將 PD2 培養基加以修改，以 potato starch 取代 PD2 培養基中之 BSA 成份，並命名為 PD3 培養，及將 PD2 培養基中之 BSA 改為 charcoal，並命名為 PD4 培

圖一、梨葉緣焦枯病病徵(A)葉片不同程度葉緣焦枯病徵(B)罹病梨樹枝條呈現葉緣焦枯病徵。

Fig. 1. Symptoms of pear leaf scorch. (A) Different degrees of scorch symptoms on leaves. (B) Leaf scorch symptoms on the twings of diseased tree.



圖二、梨葉緣焦枯病侷限導管細菌棲息梨樹組織導管內之情形及其微細構造之照片。

Fig. 2. Micrographs of xylem-limited bacteria found in pear tissues with leaf scorch and its ultrastructure. Left- (A) Cross section shows bacterial aggregate in tracheids (arrows) of leaf lateral vein tissue; and (B) longitudinal section shows total occlusion of tracheid by bacterial aggregate. (C) Cross section shows partial occlusion by bacterial aggregate and (D) strandlike structure (arrows) a associated with xylem-limited bacteria.(Bar= 3 μm). Right-Ultrathin sections shows rod-shape and ripple-walled cells (arrow) in (A) diseased tissue and (B) axenic culture. (C) Cultured bacterium by negative stain. (Bar= 0.3 μm).

養基，此兩種培養基培養效果與 PD2 培養基效果一樣佳。此外由於 JD1 系列培養基無法培養 phony peach disease 及 plum leaf scald，故 Davis 等人又針對此兩種病之病原菌開發出 PW 培養基，而同年 Wells 等人[79]亦同時針對以上兩種病之病原菌開發出組成份不同於 PW 培養基之 BCYE 培養基。爾後 Chang 和 Walker[10]也開發出另一種類型之培養基，稱為 CS20 培養基，用於分離培養造成 oak leaf scorch 之病原菌，由於 *X. fastidiosa* 屬於難分離培養之細菌，加上對此菌之生長條件所知有限，因此以往所開發出之常用培養基中皆含有未確定組成份之物質，造成了對於此菌之營養需求、代謝過程及可能之生物合成機制皆無法探討，直至 1993 年 Chang 和 Donaldson[9]才成功開發出化學組成份確定之培養基(chemically defined medium)，稱之為 XF-26 培養基，使能對此菌之營養需求有更進一步之了解。雖然過去 *X. fastidiosa* 病原菌生長需特殊成份，但亦有報告[12, 24, 44]指出 mulberry leaf scorh 和 PD 病害之有些菌株能成功在 nutrient agar(NA)上生長，似乎此二種寄主菌株營養需求較 *X. fastidiosa* 其他不同寄主菌株較不嚴苛？

梨葉緣焦枯病菌經由嫁接途徑可成功傳播至健康梨樹[48]，同時田間觀察病株為點狀散列發生，病徵常從梨樹 1 或 2 支枝條出現，可能為典型蟲媒傳播病害型態分佈？目前筆者利用 PCR 技術在罹病梨園附近採集木質部取食者昆蟲(xylem feeders)白邊大葉蟬(*Kolla paulula* (Walker, 1858))成蟲曾偵測到正反應，基因產物進一步選殖解序証實為梨葉緣焦枯病菌系(未發表資料)，有關梨葉緣焦枯病菌蟲媒傳播資料仍待深入探討？國外有關 *X. fastidiosa* 之蟲媒最早報告為 1942 年 Hewitt[29]等人發現葉蟬(leafhopper)能傳播造成 PD 之病原菌，同年 Houston[42]等人亦發現葉蟬能傳播苜蓿矮化病。1946 年 Hewitt[31]等人發現葡萄園在接近苜蓿處受 PD 感染嚴重，並隨距離漸遠而漸少，因而認為兩者為同一病原菌所感染，並可經由蟲媒而互相傳播，目前已被證實的蟲媒計有 5 種，其分類地位分別屬於半翅目(Hemiptera)、葉蟬總科(Cicadelloidea)之大葉蟬亞科(Cicadellinae)包括藍綠尖頭葉蟬 (*Graphocephala atropunctata*)[63]、褐透翅尖頭葉蟬 (*Homalodisca coagulata*)[7]、綠尖頭葉蟬(*Draeculacephala minerva*)[53]與紅尖頭葉蟬(*Carneocephala fulgida*)[30]等 4 種，以及 1 種沫蟬總科(Cercopoidea)之尖胸沫

蟬科(Aphrophoridae)黃頭長沫蟬(*Philaenus spumarius*)，例如尖頭葉蟬(sharpshooter)被認為能傳播大部份或全部 *X. fastidiosa* 之菌株，而尖頭葉蟬之寄主範圍廣[2]，包括許多草本和木本植物，常年不斷之寄主及昆蟲季節遷移性，常導致中間寄主暴露於 *X. fastidiosa* 存在之威脅，因而引起病害之大量發生。而此病原菌在蟲體內最短潛伏期為二小時或更短且能在前腸(forgut)部位增殖，但不會藉由卵傳至下一代，成蟲吸取病菌能持續傳播病菌至死亡，而若蟲獲得病菌經蛻皮後即無法成功傳播病菌[65]。

梨葉緣焦枯病菌PCR鑑定與偵測技術

有關 *X. fastidiosa* 之鑑定與偵測，早期只能以特殊培養基培養、光學顯微鏡及電子顯微鏡切片觀察[8, 40]，但耗時費力。在 80 年代後血清學技術發展日益成熟普及，加上血清學技術可以改善檢測效率及大規模樣品快速檢測，普遍應用於 *X. fastidiosa* 病原菌檢測、流行病學調查及蟲媒偵測[6, 11, 57, 62, 73, 81]。近年分子生物學的鑑定技術如核酸探針(DNA probe)[71]、聚合酵素連鎖反應(polymerase chain reaction, PCR)[56]及隨機增幅核酸多型性分析(random amplified polymorphic DNA, RAPD)[80]等具有快速、專一性及高靈敏度之特性，廣泛應用於植物病原細菌之鑑定及偵測，其中 PCR 技術普遍應用於 *X. fastidiosa* 之檢測[52, 59, 61, 62]，甚至有些已開發專一性引子對可鑑別來自不同寄主植物之 *X. fastidiosa* 菌株菌群[4, 60]。

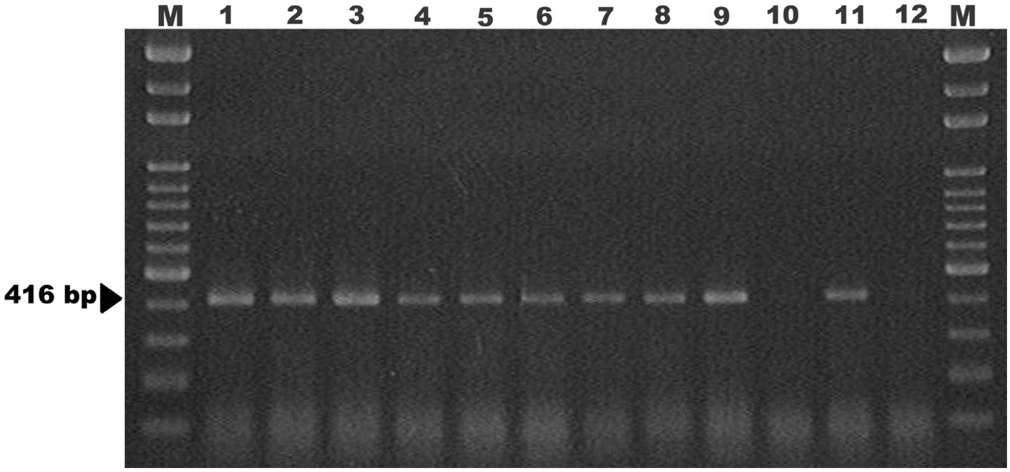
目前 *X. fastidiosa* 菌株可依交叉接種試驗資料可分為幾個菌群，例如來自於許多寄主植物之 PD 菌群、來自柑橘及咖啡菌株之 CVC 菌群及來自桃與李所分離的菌株群[66]。不同菌群目前已有開發鑑別性引子對進行鑑定，例如 Pooler 和 Hartung[60] 利用 RAPD 產物發展 PCR 引子對(CVC-1/272-2-int)，以區分 *X. fastidiosa* 中的 CVC 菌群和非 CVC 菌群的菌株；Banks 等人[4]也以 RAPD 技術發展引子對(XF176f/XF686r)可專一性鑑別 PD 菌群之菌株。

早期利用國外已設計 *X. fastidiosa* 廣效性引子對 RST31/RST33[52]及 272-1-int/272-2-int[60]測試來自國內 8 個橫山梨產區之 30 個梨葉緣焦枯病菌

株皆無法增幅出預期之基因產物片段，而過去的研究亦指出梨葉緣焦枯病菌與其他寄主植物 *X. fastidiosa* 菌株無血清相關性[46, 48]，此等結果意謂著梨葉緣焦枯病菌菌株與其他寄主植物 *X. fastidiosa* 菌株在基因層次之親緣性有明顯差異。利用隨機引子 OPA11 進行 RAPD 分析，選殖獲得梨葉緣焦枯病菌之 1,412 bp 大小 DNA 片段，核苷酸序列中 494-1,412 bp 與目前線上基因庫已註冊 DNA 序列之相似度皆高於 85%以上，而 1-493 bp 與目前線上基因庫已註冊 DNA 序列之相似度皆低於 10%，因此，1~493 bp 為梨葉緣焦枯病菌共有之特異性核苷酸序列，從中設計獲得 1 組引子對 PLS-F(5'-TGGACGTTGTGGTATCGGTG-3') / PLS-R(5'-TTGAAGTTGAC GTGTGGCTG-3') (圖三)，此引子對只對梨葉緣焦枯病菌菌株可增幅 416 bp 大小基因產物，其他 7 種寄主植物 *X. fastidiosa* 菌株及一般植物病原細菌皆無基因產物，同時 PCR 黏合溫度測試更可高達 67℃，顯示設計之引子對 PLS-F/PLS-R 對梨葉緣焦枯病菌菌株具有高度專一性，引子對可成功檢測田間採集的罹病梨樹組織(圖四)，此引子對有潛力應用在梨葉緣焦枯病之罹病組織、蟲媒及中間寄主等方面之病原菌偵測[76]。

```
1    GCACTGTTATTCCTGAGCGCATCCGCGCAATGGACGTTGTGGTATCGGTGTAGATGACGG
                                   PLS-F →
61   ATGTCATGTTCTTGATCGTCTCTAAGGTATTAAAGAACATGGAGCGGCGATACACGCTCA
121  GTACGATTGGATCAGCGATCCCCGGGTCAAATGACAACACCTATGCATCAAACGCCGTCG
181  CAACCGATAACCAGCGGCGCAAGATACATCGCTTCAAACACCATCTCTATACACCATATA
241  CGTGGGATGGACTATGACATCCGTGACAGATGTTTGCACCTGTTCCTAGAAGAACGTTGC
301  ACGCCGTTTTACCTCACGGCGTGCATGACAACCAGGTTCACAAGACTAGCCCAGTACGGC
361  ACTCATTTGGCCTCGTGATCTGACGAGATGTGTAGCTCTCGCTACCCAAGCGACTCTGGT
                   ← PLS-R
421  CCATCCCAGCCACACGTCAACTTCAAGAATCCGACCTCTTGCCCAAGGGGCGCTACAGCC
481  AAGCACCACGTACCTCAGCAACCGGGTTGCATACTGCGATTGCGAGCAAGATAGTCAGCA
```

圖三、選殖株重組質體 OPA11-1400 嵌入 DNA 片段之部分 540 bp (1-540) 核苷酸序列及引子對 PLS-F (31-50) / PLS-R (427-456)之 20 bp 核苷酸序列。
Fig. 3. The 540 bp (1-540) partial nucleotide sequence selected from the inserted DNA fragment in recombinant plasmid OPA 11-1400 and the 20 bp nucleotide sequences of primer of PLS-F (31-50) / PLS-R (427-456).



圖四、利用引子對 PLS-F/PLS-R 進行聚合酵素連鎖反應偵測田間不同罹病梨樹組織內之梨葉緣焦枯病病菌。

Fig. 4. Detection of pear leaf scorch bacteria (PLSB) of diseased tissues from different pear trees in fields by polymerase chain reaction with primer pair PLS-F/PLS-R. M: Gen-100 DNA Ladder (GeneMark, Taiwan); Lanes 1-9: samples from different pear diseased trees; Lane 10: healthy tissue; Lane 11: total DNA of PLSB as positive control; Lane 12: ddH_2O as negative control.

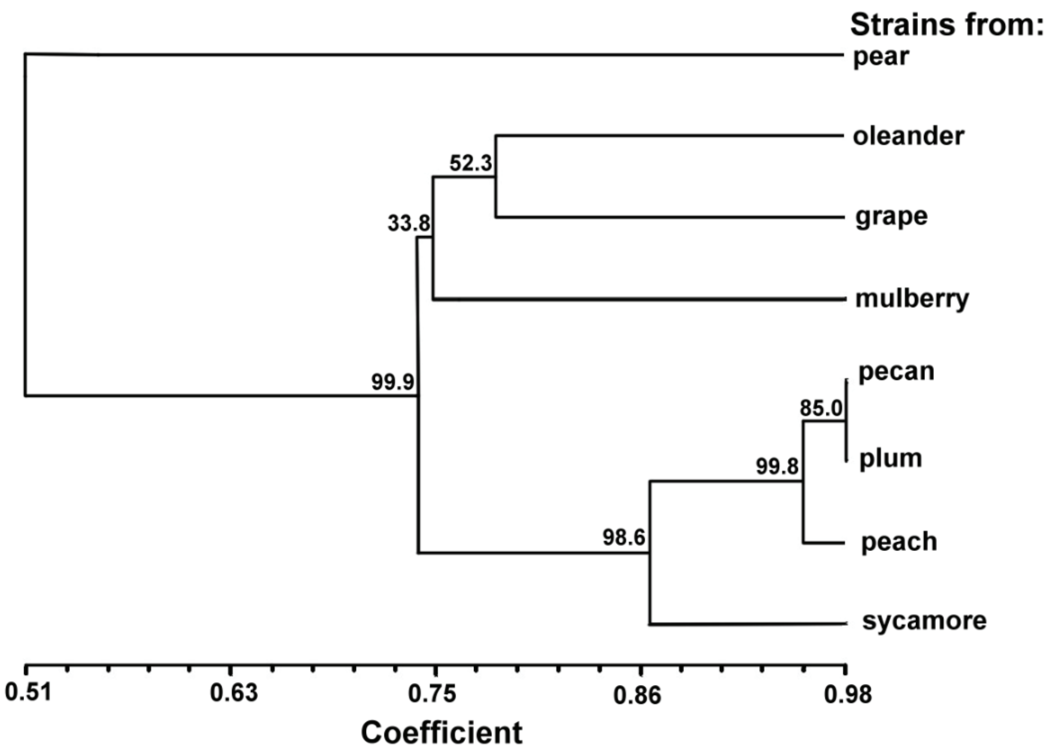
梨葉緣焦枯病菌與其他寄主植物來源菌株之親緣關係

X. fastidiosa 為寄主範圍廣之植物病原細菌，主要分佈於美洲地區，近年來美洲地區的 *X. fastidiosa* 菌株以 RAPD、ERIC-PCR 及 16S rDNA 等分子技術分析，可將不同寄主植物 *X. fastidiosa* 菌株區分為不同菌群[1, 14, 15, 16, 17, 27, 43, 51, 60, 67, 70]，而不同菌群間似乎皆存有病原性、培養特性及分子特徵差異性。有些學者推測不同 *X. fastidiosa* 菌群的形成可能與寄主植物之特化性有關[35, 37, 40]。

應用 RAPD、ERIC-PCR 及 BOX-PCR 三種 DNA 指紋圖譜及親緣樹狀圖分析結果發現國內梨葉緣焦枯病菌為獨立菌群，與其他 7 種寄主植物 *X. fastidiosa* 菌株親緣相關性低，這些差異性顯示台灣梨葉緣焦枯病菌是 *X. fastidiosa* 菌群中一獨立菌群，而 7 種其他寄主植物 *X. fastidiosa* 菌株彼此間

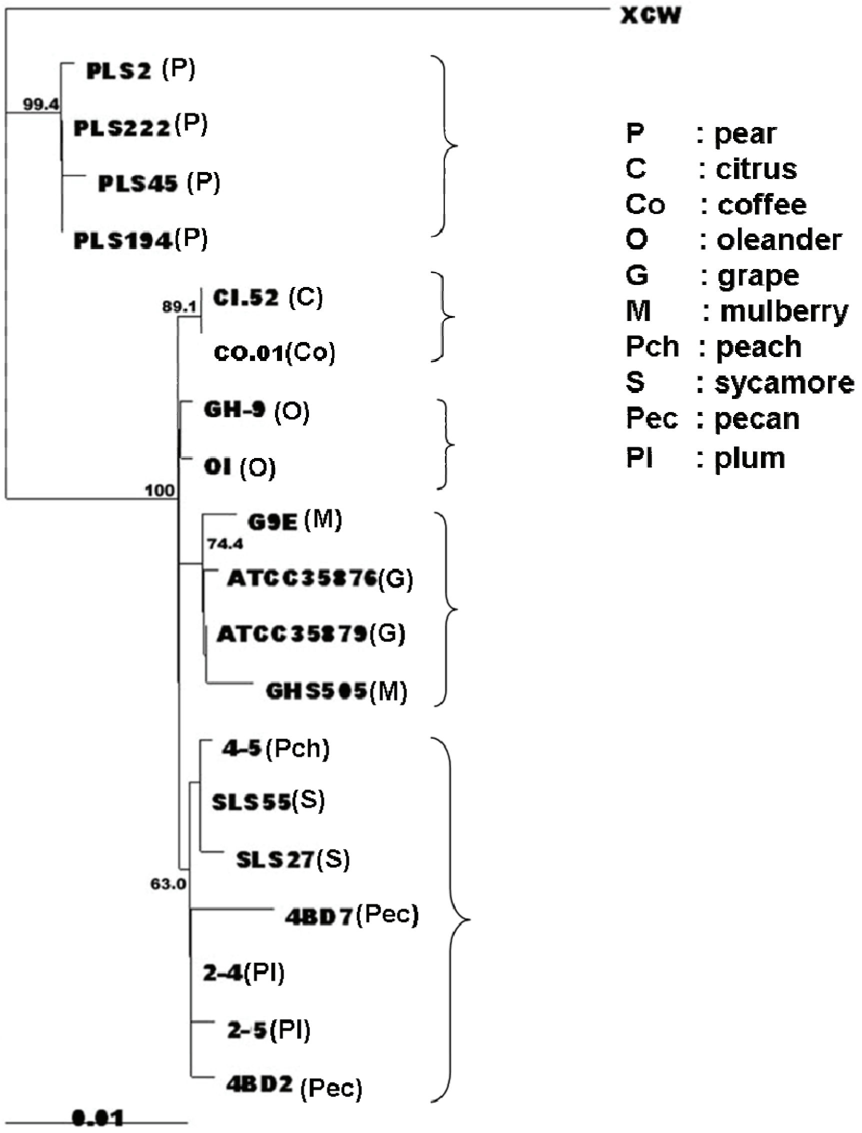
有親緣相關性 (coefficient＞0.70)，可區分為 3 個菌群，分別為：(1)夾竹桃及葡萄菌群、(2)桑椹菌群及(3)胡桃樹、李樹、桃樹及無花果菌群(圖五)[75]，此結果與國外文獻報導相似，例如 Pooler 等人以 RAPD 技術將測試 7 種寄主植物 *X. fastidiosa* 菌株區分為 5 群[60]，分別為：(1)柑橘菌群、(2)李及榆樹菌群、(3)葡萄及豬草(ragweed)菌群、(4)杏仁菌群及(5)桑椹菌群；Qin 等人[67]利用 ERIC-PCR 及 RAPD 技術對來自北美地區及南美地區共 9 個不同寄主植物 57 個 *X. fastidiosa* 菌株進行比較分析，可將試驗菌株區分為北美 7 種寄主植物菌群及南美柑橘及咖啡菌群，其中北美菌群可細分為 3 個菌群，分別為：(1)桑椹菌群、(2)葡萄、豬草及杏仁菌群及(3)榆樹、橡樹及李樹菌群。另由三種 DNA 指紋圖譜技術分析結果示國內 8 個橫山梨產區之 30 個梨葉緣焦枯病菌菌株間無差異性，彼此間具同源性，此結果顯示國內不同地區之梨葉緣焦枯病菌菌株可能來自於同一起源。

筆者進一步分析梨葉緣焦枯病菌及來自於 9 種其他寄主植物 *X. fastidiosa* 菌株之 16S rRNA 基因及 16S-23S rRNA 基因區間序列，根據樹型關係遠近皆可將 10 種寄主植物 *X. fastidiosa* 區分為 2 群分別為梨葉緣焦枯病菌菌群及其他寄主植物 *X. fastidiosa* 菌群，這 2 群 bootstrap 值都在 90％以上，其他寄主植物 *X. fastidiosa* 菌群中可再細分為 4 個次菌群分別為：(1)夾竹桃菌群、(2)葡萄與桑椹菌群、(3)咖啡與柑橘菌群及(4)胡桃樹、李樹、桃樹及無花果菌群(圖六及圖七)。其中葡萄菌系，柑橘菌系，李樹、桃樹及無花果菌系分析結果皆分別符合 Schaad 等人定義的 3 個亞種，但梨葉緣焦枯病菌菌群與其他寄主植物 *X. fastidiosa* 菌株間於 16S-23S rRNA 基因區間序列分析相似值介於 87.4~87.6％遠低於 *X. fastidiosa* 3 個亞種間相似值介於 97.9~99.2％[72]；另 Mehta 和 Rosato[51]研究中亦發現梨葉緣焦枯病菌(1 個菌株由筆者提供)與柑橘 *X. fastidiosa* 菌株進行 DNA-DNA 雜合試驗 (hybridization)，彼此間為同源性低於 20％，綜合過去研究資料及筆者分析結果，推論台灣梨葉緣焦枯病菌菌群與其他寄主植物 *X. fastidiosa* 菌群間應為亞種以上的層級差異或可提升為 *Xylella* 屬中一新種。



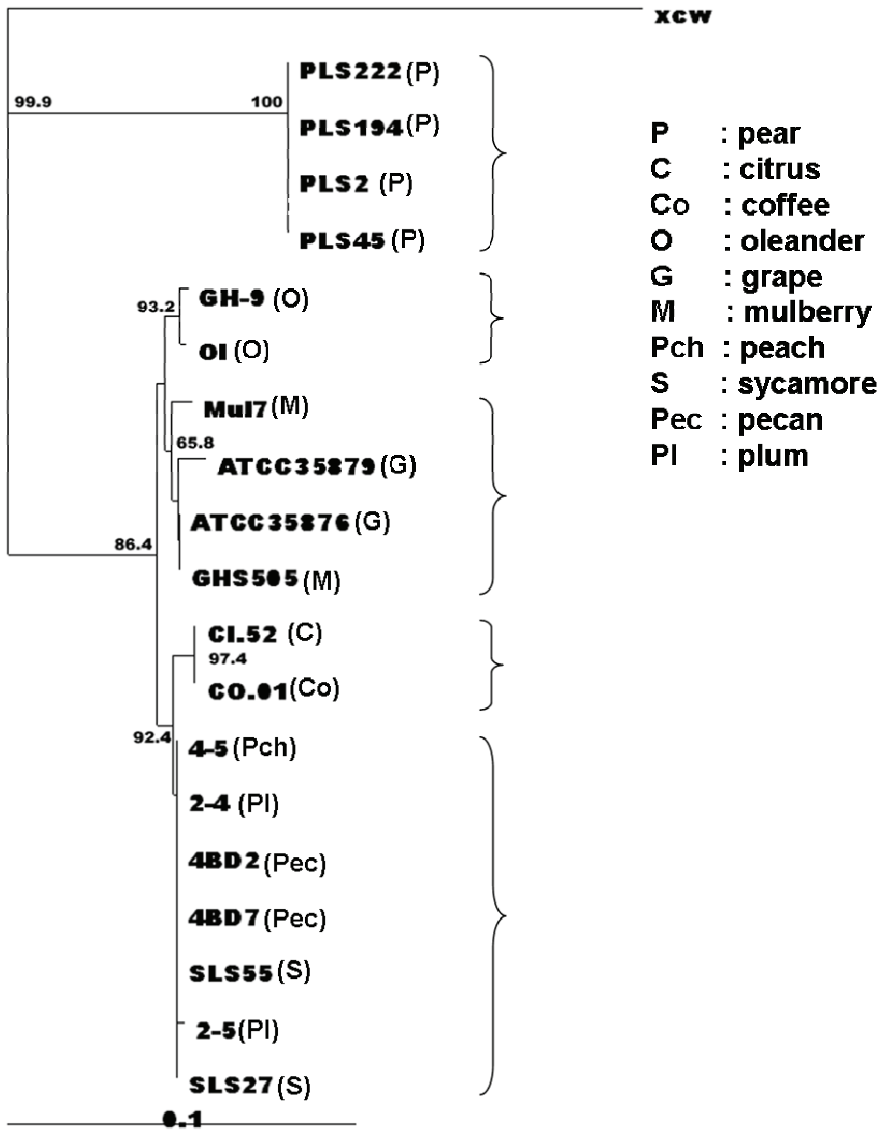
圖五、分析梨葉緣焦枯病菌與國外不同寄主植物 *Xylella fastidiosa* 菌株之 RAPD、ERIC-PCR 及 BOX-PCR DNA 指紋圖譜條帶，並經由 NTSYS-pc version 2.0 電腦軟體中的 UPGMA 程式繪製相似值樹狀圖。另由 WinBoot 程式計算出各群集之信賴界限，標示於親緣樹狀圖中各群集之轉折點。

Fig. 5. A dendrogram showing the genetic relationships between strains of pear leaf scorch bacterium and strains of *Xylella fastidiosa* from other hosts based on DNA fingerprintings generated by RAPD、ERIC-PCR and BOX-PCR. Data were analyzed by NTSYS-pc version 2.0 using unweighted pair-group method with arithmetic averages (UPGMA) and confirmed by bootstrapping using WinBoot program.



圖六、梨葉緣焦枯病菌與其他寄主植物 *Xylella fastidiosa* 之 16S rRNA 基因序列經由 Neighbor-joining (NJ) 方法分析親緣演化樹；枝條上的數字代表 boostrap 值。

Fig. 6. A phylogenetic tree produced with the Neighbor-joining method based on the 16S rRNA gene sequences of pear leaf scorch bacteria and strains of *Xylella fastidiosa* from other hosts. The Numbers at the selected nodes indicate the levels of bootstrap support (percentage) based on 1000 re-sampled data sets (only values greater than 60% shown). The scale bar represents 1% of sequence divergence.



圖七、梨葉緣焦枯病菌與其他寄主植物 *Xylella fastidiosa* 之 16S-23S rRNA 基因區間序列經由 Neighbor-joining (NJ) 方法分析親緣演化樹；枝條上的數字代表 boostrap 值。

Fig. 7. A phylogenetic tree produced with the Neighbor-joining method based on the 16-23S rRNA intergenic spacer of pear leaf scorch bacteria and strains of *Xylella fastidiosa* from other hosts. The Numbers at the selected nodes indicate the levels of bootstrap support (percentage) based on 1000 re-sampled data sets (only values greater than 60％ shown). The scale bar represents 1％ of sequence divergence.

梨葉緣焦枯病防治策略

X. fastidiosa 引起了許多植物之病害，除少數病害像 PD 病害和 phony peach disease 外，大部分皆被認定為較弱之病菌，許多 *X. fastidiosa* 之菌株引起之病害常為慢性衰弱或無表現病徵之狀態存在，病菌長久棲息聚集在導管，當寄主弱勢或環境壓力不利寄主，才明顯表現出病徵，包括乾旱、其他複合病害、機械性斷根、過度生產、果實熟化和生理老化(34, 41)，許多寄主直至果實成熟或晚秋生理老化，病徵才表現，另外生理老化實際影響寄主黃化、落葉及葉柄尖化病徵之表現。

梨葉緣焦枯病田間觀察亦顯示樹木年老衰落，長久缺水及過量生產及土壤惡化等因素下較容易表現出病徵，因此規劃合理管理栽培制度可適度減輕病株之嚴重度，而維持其基本生產力應可期待。化學治療方面，PD 病害和 plum leaf scald 均顯示注射四環黴素能減輕病勢之發展及病徵之表現(13, 38)，臺灣梨葉緣焦枯病每年 5 月和 10 月各注射四環黴素亦顯示能恢復樹勢之活力，同時注射後病株之徒長枝數較無注射之病株有明顯之增加(48)，然此法治標不治本，只能暫時降低病株病菌之族群，假如能配合增施優良有機肥或其他管理措施，將能增加植物之抵抗性，提高防治之效益，藥劑之注射時機應選在病株內細菌族群將增加之 5 月及病菌族群達高峰之 10 月，因 5 月果實漸成熟導致病徵逐漸呈現和 10 月梨樹生理老化會加速落葉影響光合作用減少樹液之回流，選擇此二階段注射藥劑，應是最理想之用藥時機。綜合前面所述擬定梨葉緣焦枯病之防治策略，其方法如下：

1. 禁止從病株採穗或枝條供繁殖用。
2. 嚴重之病株應砍除，同時不具生產力之小樹如罹病亦應立即淘汰。
3. 部份具病徵之成樹枝幹必須鋸除。
4. 定期噴灑殺蟲劑消滅田間蟲媒及鏟除中間寄主植物如雜草(待深入探討證實？)。
5. 輕微病株在 5 月及 10 月注射四環黴素。
6. 梨果收獲後增施發酵充份之優良有機肥。
7. 果園充份施水，防止乾旱發生。

結　論

X. fastidiosa 為國際重要檢疫病原細菌，可為害許多經濟作物，造成重大經濟損失，例如 PD 於 1892 年首次發現在美國加州南部地區[36]，爾後被命名為加州葡萄病(Califonia vine disease)，至今一直是美國東南地區葡萄產區之風土病 (endemic)，亦為該地區葡萄產業之主要限制因子，影響釀酒工業之葡萄栽培及生產甚鉅，PD 亦發現在中美洲和南美州地區造成危害，除美洲地區外，歐盟國家如南斯拉夫 Kosova 地區為首次被記錄 PD 危害之地區[5];PD 在加州許多葡萄栽植區域之葡萄園每年皆會發生，在 Napa、Sonoma 及 Mendocina 等郡之商業化栽植之葡萄園皆嚴重發生，一般認為該區域 PD 之流行發生與其蟲媒族群之建立有密切關係[64]。PD 亦會影響畜牧之產業，PD 之病原會引起苜蓿矮化病，造成苜蓿之嚴重減產[31]。PD 在加州曾藉由人為攜入而發生，本病可能廣泛分佈於加州地區或其他國家葡萄產區而未被發現，主要受限於該區域之寄主範圍仍未完全被發現及各國間進一步檢測 PD 存在之計畫仍未完全實行。本文詳細論述台灣梨葉緣焦枯病徵學、病原菌特性、PCR 鑑定與偵測技術、梨葉緣焦枯病菌與其他寄主植物來源菌株之親緣關係及防治策略，期望國內梨產業生產者瞭解本病生態學基本資料，能有效降低病害發生，但有關本病之蟲媒及中間寄主植物資料仍欠缺，未來企盼會引起許多學者興趣，深入此課題領域之研究；另本文論述 *X. fastidiosa* 菌株之 PCR 相關鑑定技術亦可提供動植物防檢局進行實務檢疫上之應用，防杜國外其他寄主植物 *X. fastidiosa* 菌株可能藉由種苗植物入侵台灣之風險。

引用文獻

1. Albibi, R., Chen, J., Lamikanra, O., Banks, D., Jarret, R. L., and Smith, B. J. 1998. RAPD fingerprinting *Xylella fastidiosa* Pierce’s disease strains isolated from vineyard in North Florida. FEMS Microbiol. Letters 165:347-352.

2. Aldlerz, W. C. 1980. Ecological observation on two leaf hopper that transmit the Pierce's disease bacterium. Proc. Fla. State Hortic. Soc. 93:115-120.
3. Anderson, P. C., and French, W. J. 1987. Biophysical characteristics of peach trees infected white phony peach disease. Physiol. M. L. Plant Pathol. 31:25-40.
4. Banks, D., Albibi, R., Chen, J., Lamikanra, O., Jarret, R. L., and Smith, B. J. 1999. Specific detection of *Xylella fastidiosa* Pierce's disease strains. Curr. Microbiol. 39:85-88.
5. Berisha, B., Chen, Y. D., Zhand, G. Y. and Chen, T. A. 1998. Isolation of Pierce's disease bacteria from grapevines in Europe. Eur. J. Plant Pathol. 104:427-433.
6. Brlansky, R. H., Lee, R. F., Timmer, L. W., Purcifull, D. E., and Raju, B. C. 1982. Immunofluorescent detection of xylem-limited bacteria *in situ*. Phytopathology 71:1444-1448.
7. Brlansky, R. H., Timmer, L. W., French, W. J., and McCoy, R. E. 1983. Colonization of the sharpshooter vectors, *Oncometopia nigricans* and *Honalodisca coagulata*, by xylem-limited bacteria. Phytopathology 73:530-535.
8. Chagas, C. M., Rossetti, V., and Beretta, J. G. 1992. Electron microscopy studies of a xylem-limited bacterium in sweet orange affected with citrus variegated chlorosis disease in Brazil. J. Phytopathol. 134:306-312.
9. Chang, C. J., and Donaldson, R. C. 1993. *Xylella fastidiosa*: cultivation in chemically defined medium. Phytopathology 83:192-194.
10. Chang, C. J., and Walker, J. T. 1988. Bacterial leaf scorch of northern red oak: isolation, cultivation, and pathogenicity of a xylem-limited bacterium. Plant Dis. 72:730-733.
11. Chang, C. J., Garnier, M., Zreik, L., Rossetti, V., and Bove, J. M. 1993. Culture and serological detection of the xylem-limited bacterium causing

citrus variegated chlorosis and its identification as a strain of *Xylella fastidiosa*. Curr. Microbiol. 27:137-142.

12. Chang, C. J., Robacker, C. D., and Lane, R. P. 1990. Further evidence for the isolation of *Xylella fastidiosa* on nutrient agar from grapevines showing Pierce’s disease symptoms. Can. J. Plant Pathol. 12:405-408.
13. Chang, C. J., Yonce, C. E., and Gardner, D. 1987. Suppression of leaf scald symptom in plum by oxytetracycline injection. Ann. Phytopath. Soc. Japan 53:354-359.
14. Chen, J., Banks, D., Jarret, R. L., Chang, C. J., and Smith, B. J. 2000. Use of 16S rDNA sequences as signature characters to identify *Xylella fastidiosa*. Curr. Microbiol. 40:29-33.
15. Chen, J., Chang, C. J., Jarret, R. L., and Gawel, L. 1992. Genetic variation among *Xylella fastidiosa* strains. Phytopathology 82:973-977.
16. Chen, J., Hartung, J. S., Chang, C. J., and Vidaver, A. K. 2002. An evolutionary perspective of Pierce's disease of grapevine, citrus variegated chlorosis, and mulberry leaf scorch diseases. Curr. Microbiol. 45:423-428.
17. Chen, J., Jarret, R. L., Qin, X., Hartung, J. S., Banks, D., Chang, C. J., and Hopkins, D. L. 2000. 16S rDNA sequence analysis of *Xylella fastidiosa* strains. System. Appl. Microbiol. 23:349-354.
18. Davis, M. J., and Purcell, A. H. 1978. Pierce’s disease of grapevines: isolation of the causal bacterium. Science 199:75-77.
19. Davis, M. J., French, W. J., and Schaad, N. W. 1981. Axenic culture of the bacteria associated with phony disease of peach and plum leaf scald. Curr. Microbiol. 6:309-314.
20. Davis, M. J., Purcell, A. H., and Thomson, S. V. 1980. Isolation medium for the Pierce’s disease bacterium. Phytopathology. 70:425-429.
21. de Lima, J. E. O., Miranda, V. S., Hartung, J. S., Brlansky, R. H., Coutinho, A., Roberto, S. R., and Carlos, E. F. 1998. Coffee leaf scorch bacterium: Axenic culture, pathogenicity, and comparison with *Xylella fastidiosa* of

citrus. Plant Dis. 82:94-97.

22. Evert, D. R. 1987. Influence of phony disease of peach on stem hydraulic conductivity and leaf xylem pressure potential. J. Amer. Soc. Hortic. Sic. 112:1032-1036.
23. French, W. J., and Stassi, D. L. 1978. Response of phony-infected peach trees to gibberellic acid. Hort. Sci. 13:158-159.
24. Fry, S. M., Milholland, R. D., and Huang, P. Y. 1990. Isolation and growth of strains of *Xylella fastidiosa* from infected grapevines on nutrient agar media. Plant Dis. 74:522-524.
25. Goheen, A. C., Nyland, G., and Lowe, S. K. 1973. Association of a rickettsia-like organism with Pierce's of grapevines and alfalfa dwarf and heat therapy of the disease in grapevines. Phytopathology 63:341-345.
26. Hearon, S. S., Sherald, J. L., and Kostka, S. J. 1980. Association of xylem-limited bacteria with elm, sycamore and oak leaf scorch. Can. J. Bot. 58:1986-1996.
27. Hendson, M., Purcell, A. H., Chen, D., Smart, C., Guilhabert, M., and Kirkpatrick, B. 2001. Genetic diversity of Pierce's disease strains and other pathotypes of *Xylella fastidiosa*. Appl. Environ. Microbiol. 67:895-903.
28. Hewitt, W. B., and Houston B. R. 1941. Association of Pierce's disease of grapevine and alfalfa dwarf in California. Plant Dis. Rep. 25:475-476.
29. Hewitt, W. B., Frazier, N. W., and Houston, B. R. 1942. Transmission of Pierce's disease of grapevines with leaf hopper. (Abstr.) Phytopathology 32:8.
30. Hewitt, W. B., Frazier, N. W., Jacob, H. E., and Freytag, J. H. 1942. Pierce's disease of grapevines. Calif. Agric. Exp. Stn. Circ. 353: 1-32.
31. Hewitt, W. B., Houston B. R., Frazier, N. W., and Freitag, J. H. 1946. Leafhopper transmission of the virus causing Pierce's disease of grape and dwarf of alfalfa. Phytopathology 36:117-128.
32. Hewitt, W. B., Houston, B. R., Frazier, N. W., and Freitag, J. H., 1946.

Pierce’s disease of grapevine. Calif. Agric. Exp. Stn. Circ. 353:1-32.

33. Hopkins, D. L. 1981. Seasonal concentration of the Pierce’s disease bacterium in grapevine stems petioles, and leaf veins. Phytopathology 71:415-418.
34. Hopkins, D. L. 1985. Effects of plant growth regulators on development of Pierce’s disease symptoms in grapevine. Plant Dis. 69:944-946.
35. Hopkins, D. L. 1989. *Xylella fastidiosa*: xylem-limited bacterial pathogen of plants. Annu. Rev. Phytopathol. 27:271-290.
36. Hopkins. D. L. 2001. Pierce’s disease. In: *Encyclopaedia of Plant Pathology Volume II,* Maloy, O.C. and Murray, T.D.(eds), John Wiley and Sone Inc., New York, pp71-772.
37. Hopkins, D. L., and Adlerz, W. C. 1988. Natural hosts of *Xylella fastidiosa* in Florida. Plant Dis. 72:492-431.
38. Hopkins, D. L., and Mollenhauer, H. H. 1973. Rickettsia-like bacterium associated with Pierce’s disease of grapes. Science 179:298-300.
39. Hopkins, D. L., and Mortensen, J. A. 1971. Suppression of Pierce’s disease symptoms by tetracycline antibiotics. Plant Dis. Rep. 55:610-612.
40. Hopkins, D. L., and Purcell, A. H. 2002. *Xylella fastidiosa* : cause of Pierce's disease of grapevine and other emergent diseases. Plant Dis. 86: 1056-1066.
41. Hopkins, D. L., and Thompson, C. M. 1984. Seasonal concentration of Pierce’s disease bacterium in “Carlos” and “Welder” muscadine grape compared with “Schuyler” bunch grape. Hort. Science 19:419-420.
42. Houston, B. R., Frazier, N. W., and Hewitt, W. B. 1942. Leaf-hopper transmission of the alfalfa dwarf virus. (Abstr.) Phytopathology 32:10.
43. Kamper, S. M., French, W. J., and Dekloet, S. R. 1985. Genetic relationships of some fastidious xylem-limited bacteria. Int. J. Syst. Bacteriol. 35:185-188.
44. Kostka, S. J., Tattar, T. A., Sherald, J. L., and Hurtt, S. S. 1986. Mulberry

leaf scorch, a new disease caused by a fastidious xylem-inhabiting bacterium. Plant Dis. 70:690-693.

45. Lee, R. F., Raju, B. C., Nyland, G., and Goheen, A. C. 1982. Phytotoxin(s) produced in culture by the Pierce's disease bacterium. Phytopathology 72:886-888.

46. Leu, H. H., Leu, L. S., and Lin, C. P. 1998. Development and application of monoclonal antibodies against the causal bacterium of pear leaf scorch, *Xylella fastidiosa*. J. Phytopathol. 146:31-37.

47. Leu, L. S., and Su, C. C. 1990. Preliminary report on pear leaf scorch induced by xylem-limited bacteria in Taiwan. (Abstr.) Plant Prot. Bull. 32:329.

48. Leu, L. S. and Su, C. C. 1993. Isolation, cultivation, and pathogenicity of *Xylella fastidiosa,* the causal bacterium of pear leaf scorch disease in Taiwan. Plant Dis. 77:642-646.

49. Liaw, W. J. 2005. The development of pear cultivation technique in Taiwan. p.47-54. Proceedings of a Symposium on Cultural Techniques and Management in Pear. 510pp. (in Chinese).

50. Lin, J. H., Liaw, W. J., Lin, H. S., and Chang, L. R. 1991. The review and outlook of pear cultivation in Taiwan. p.379-396. Proceeding of A Symposium on Fruit Production, Research and Development in Taiwan. 429pp. (in Chinese).

51. Mehta, A. and Rosato, Y. B. 2001. Phylogenetic relationships of *Xylella fastidiosa* strains from different hosts, based on 16S rDNA and 16-23S intergenic spacer sequences. Int. J. Syst. Evol. Microbiol. 51:311-318.

52. Minsavage, G. V., Thompson C. M., Hopkins, D. L., Leite, R. M. V. B. C., and Stall, R. E. 1994. Development of polymerase chain reaction protocol for detection of *Xylella fastidiosa* in plant tissue. Phytopathology 84:456-461.

53. Mircetich, S. M., Lowe, S, K., Moller, W. J., and Nyland, G. 1976. Etiology

of almond leaf scorch disease and transmission of the causal agent. Phytopathology 66:17-24.

54. Mollenhauer, H. H., and Hopkins, D. L. 1974. Ultrastructural study of Pierce's disease bacterium in grape xylem tissue. J. Bacteriol. 119:612-618.
55. Mollenhauer, H. H., and Hopkins, D. L. 1976. Xylem morphology of Pierce's disease-infected grapevines with different levels of tolerance. Physiol. Plant Pathol. 9:95-100.
56. Mullis, K. B., and Falloona, F. A. 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalysed chain reaction. Methods Enzymol. 155:335-350.
57. Nomé, S. F., Raju, B. C., Goheen, A. C., Nyland, G., and Docampo, D. 1980. Enzyme-linked immunosorbent assay for Pierce's disease bacteria in plant tissues. Phytopathology 70:746-749.
58. Nyland, G., Goheen, A. C., Lowe, S. K., and Kirkpatrick, H. C. 1973. The ultrastructure of a rickettsia-like organism from a peach tree affected with phony disease. Phytopathology 63:1275-1278.
59. Oliveira, A. C., Vallim, M. A., Semighini, C. P., Araújo, W. L., Goldman, G. H., and Machado, M. A. 2002. Quantification of *Xylella fastidiosa* from citrus trees by real-time polymerase chain reaction assay. Phytopathology 92:1048-1054.
60. Pooler, M. R., and Hartung, J. S. 1995. Genetic relationships among strains of *Xylella fastidiosa* from RAPD-PCR data. Curr. Microbiol. 31:134-137.
61. Pooler, M. R., and Hartung, J. S. 1995. Specific PCR detection and identification of *Xylella fastidiosa* strains causing citrus variegated chlorosis. Curr. Microbiol. 31:377-381.
62. Pooler, M. R., Myung, A. S., Bentz, J., Sherald J., and Hartung J. S. 1997. Detection of *Xylella fastidiosa* in potential insect vector by immunomagnetic separation and nested polymerase chain reaction. Letters Appl. Microbiol. 25:123-126.

63. Purcell, A. H. 1975. Role of the bluegreen sharpshooter, *Hordnia circellata,* in the epidemiology of Pierce's disease of grapevines. Environ. Entomol. 4: 745-752.

64. Purcell, A. H. 2000. Glassy-winged sharpshooter (*Homalodisca coagulata*) http://nature.berkeley.edu/xylella/oss.html

65. Purcell, A. H., and Finlay, A. H. 1979. Evidence for noncirculative transmission of Pierce's disease bacterium by sharpshooter leaf hoppers. Phytopathology 69:393-395.

66. Purcell, A. H., and Hopkins, D. L. 1996. Fastidious xylem-limited bacterial plant pathogens. Annu. Rev. Phytopathol. 34:131-151.

67. Qin, X., Miranda, V. S., Machado, M. A., Lemos, E. G. M., and Hartung, L. S. 2001. An evaluation of the genetic diversity of *Xylella fastidiosa* isolated from diseased citrus and coffee in São Paulo, Brazil. Phytopathology 91:599-605.

68. Raju, B. C., and Wells, J. M. 1986. Disease caused by fastidious xylem-limited bacteria and strategies for management. Plant Dis. 70:182-186.

69. Raju, B. C., Wells, J. M., Nyland, G., Brlansky, R. H., and Lowe, S. K. 1982. Plum leaf scald: isolation, culture, and pathogenicity of the causal agent. Phytopathology 72:1460-1466.

70. Rosato, Y. B., Neto, J. B., Miranda, V. S., Carlos, E. F., and Manfio, C. P. 1998. Diversity of a *Xylella fastidiosa* population isolated from *Citrus sinensis* affected by citrus variegated chlorosis in Brazil. System. Appl. Microbiol. 21:593-598.

71. Schaad, N. W., Cheng, S. S., Tamski, S., Hatziloukas, E., and Panopoulos, N. J. 1995. Identification of *Pseudomonas syringae* pv. *phaseolicola* by DNA hybridization probe. Phytopathology 79:903-907.

72. Schaad, N. W., Postnikova, E., Lacy, G., Fatmi, M., and Chang, C. J. 2004. *Xylella fastidiosa* subspecies: *X. fastidiosa* subsp. *piercei*, subsp. nov., *X. fastidiosa* subsp. *multiplex* subsp. nov., and *X. fastidiosa* subsp. *pauca* subsp. nov. Syst. Appl. Microbiol. 21:290-300.

73. Sheraid, J. L., and Lei, J. D. 1991. Evaluation of a rapid ELISA test kit for detection of *Xylella fastidiosa* in landscape. Plant Dis. 75:200-203.

74. Su, C. C. and Leu, L. S. 1995. Distribution of pear leaf scorch and monthly isolation of its causal organism, *Xylella fastidiosa* from infected trees. Plant Pathol. Bull. 4:30-33.

75. Su, C. C., Yang, W. J., Feng, C. Y., Hsu, S. T., and Tzeng, K. C. 2008. The application of DNA fingerprintings amplified by arbitrary primers in differentiating pear leaf scorch bacterium from other *Xylella fastidiosa* strains. Plant Pathol. Bull. 17: 261-269.

76. Su, C. C., Yang, W. J., Hsu, S. T., and Tzeng, K. C. 2008. Specific detection of *Xylella fastidiosa* strains causing pear leaf scorch by polymerase chain reaction. Plant Pathol. Bull. 17: 183-194.

77. Wells, J. M., Raju, B. C., and Nyland, G. 1983. Isolation, culture, and pathogenicity of the bacterium causing phony disease of peach. Phytopathology 73:859-862.

78. Wells, J. M., Raju, B. C., Hung, H. Y., Weisburg, W. G., Mandelco-Paul, L., and Brenner, D. J. 1987. *Xylella fastidiosa* gen. nov. sp. nov.: Gram-negative xylem-limited, fastidious plant bacteria related to *Xanthomaonas* spp. Int. J. syst. Bacteriol. 36:136-143.

79. Wells, J. M., Raju, B. C., Nyland, G., and Lowe, S. K. 1981. Medium for isolation and growth of bacteria associated with plum leaf scald and phony peach diseases. Appl. Environ. Microbiol. 42:357-363.

80. Williams, J. G. K., Kubelik, A. R., Livak, K. J., Rafalski, J. A., and Tingey, S.V. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18:6531-6535.

81. Yonce, C. E. and Chang, C. J. 1987. Detection of xylem-limited bacteria from sharpshooter leafhoppers and their feeding hosts in peach environs monitored by culture isolations and ELISA techniques. Environ. Entomol. 16:68-71.

ABSTRACT

Pear leaf scorch

Su, C. C.[1, 3], Feng, C. Y.[1], Kao, C. W.[1], Tzeng, K. C.[2], and Hsu, S. T.[2]

[1] Taiwan Agricultural Chemicals and Toxic Substances Reasearch Institute, Council of Agriculture, Executive Yuan, Thaichung, Wu-feng 413, Taiwan, R.O.C.

[2] Department of Plant Pathology, National Chung Hsing University, Taichung, Taiwan, R.O.C.

[3] Corresponding author, Email: auba@tactri.gov.tw

Pear leaf scorch (PLS) disease, the only *Xylella fastidiosa*-induced disease reported from Taiwan, is commonly found in areas where the variety Hengshan (*Pyrus pyrifolia*) is grown. Strains of PLS bacterium shares similarities to strains of *X. fastidiosa* from other host origins in the requirement of complex medium and the rippled cell wall in cell structure. However, PLS strains are not serologically related to strains of *X. fastidiosa* from other hosts. Likewise, no DNA fragment was amplified from PLS strains with two general primer sets of RST31/RST33 and 272-int/272-int-2 used to detect strains of *X. fastidiosa* from other hosts. A fragment of 1412 bp in size amplified for PLS strains with a random primer OPA11 by random amplified polymorphic DNA(RAPD) technique was identified as specific for PLS strains. A set of specific primer PLS-F/PLS-R amplified a 416 bp fragment was designed from the sequence data for PLS strains. The PLS bacterium could be detected by using the specific primer set in PLS diseased leaf tissues collected from fields.The DNA fingerprintings generated by RAPD-PCR, enterobacterial repetitive intergenic consensus sequence-PCR (ERIC-PCR) and BOX-PCR of all strains of *X. fastidiosa* from eight hosts. The constructed dendrograms showed that all strains of PLS bacterium were clusted together and separate from other *X. fastidiosa* strains, and the latter wereclustered into three groups: one consisted of mulberry strains, another consisted of grape and oleanderstrains, and the third comprised pecan, plum, peach and sycamore strains. Our results indicate that the DNA

fingerprintings amplified by arbitrary primers are useful to differentiate pear leaf scorchbacteria from other *X. fastidiosa* strains. Further compared the phylogenetic relationship based on 16S rRNA sequence and 16S-23S rRNA spacer region of various strains from ten hosts using neighbor joining method. All strains of *X. fastidiosa* from different host were distinguished two groups, pear group and other host group. The other host group were subdivided into four subgroup: (1)oleander, (2)grape and mulberry, (3)citrus and coffee, and (4) pecan, peach, plum and sycamore. According to the above 16S rRNA sequence and 16S-23S rRNA spacer region data, pear leaf scorch strains were much low homology in comparison with strains of X. fastidiosa from other host and induced disease which only reported in Taiwan. We postulated pear leaf scorch strains could be a new subspecies or species within the genera of *Xylella*. The developed PCR identified techniques of *X. fastidiosa* could be a useful tool for rapid detection of PLS bacterium in insect vectors and alternative hosts in Taiwan in the future. The rational cultural pratices, such as roguing diseased plant, avoiding bud or cutting from infected trees for vegetable reproduction and increasing amendments of organic matters, could alleviate disease symptoms. Injection of oxytetracycline solution in May and October partially supressed disease development.

Key words： pear leaf scorch, *Xylella fastidiosa*, polymerase chain reaction, detection, genomic fingerprinting, 16S rRNA gene sequence, 16S-23S rRNA intergenic spacer region sequence, phylogenetic relationship